U. of Texas Southwestern Medical School.

UNPUBLISHED PRELIMINARY DATA

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I. During the months of July and August the entire laboratory and its personnel were moved to a new location. This new location is in the Woodlawn Hospital, 3819 Maple Avenue, Dallas, Texas. This hospital is a member of the Dallas County Hospital District and serves as a chronic chest disease hospital. It is under the medical direction of Dr. Charles LeMaistre, Professor of Medicine, Department of Medicine, The University of Texas Southwestern Medical School. The clinical laboratories of the hospital are under the direction of Dr. R. C. Reynolds, Assistant Professor of Pathology, Department of Pathology, The University of Texas Southwestern Medical School. Dr. Reynolds is collaborator in many of the aspects of cellular research carried on in the research laboratory. The reason for this move from The University of Texas Southwestern Medical School to the new location was to acquire additional space. In the new location the laboratory has approximately 5,000 square feet of research space arranged as follows: (1) Electron Microscopy - This area includes a complete photographic dark room, a Hitachi electron microscope, and a laboratory for fixation, embedding and cutting of cellular specimens; (2) A chemistry and cytochemistry laboratory with equipment for conventional tissue preparation, cryostat tissue sectioning and subsequent histochemical treatment; (3) A tissue culture laboratory with a separate small sterile room for culture preparation and an adjacent room for incubators and glassware cleaning; (4) A small laboratory for radioautography to be used in conjunction with tissue sectioning, tissue culture and electron microscopy; (5) A mechanical workshop for metal and wood work; (6) An electronic shop; (7) An optical electronic shop equipped with an optical bench; (8) A special instrumentation room in which are housed the ultraviolet flying spot television microscope, the ultraviolet microbeam phase contrast television

microscope, and the stepping spot microscope; (9) A time lapse photographic laboratory equipped with three phase contrast time lapse motion picture units; (10) Four office areas; and (11) A film library and projection room for the study of time lapse motion pictures, photomicrographs and electron micrographs.

The mechanical process of moving coupled with the installation of new equipment such as the electron microscope has badly disrupted the continuity of the laboratory research work. It is anticipated that the former level of research work will not be achieved for a period of two additional months. This is occasioned by the time required to rearrange all of the laboratory equipment and to integrate the new equipment into the structure of the laboratory. In the process one new technician is being trained. The entire group now consists of two senior pathologists, three electrical engineers, three biological technicians, and six part-time medical student assistants.

II. Gravitation Studies

A. As previously noted bacteria subjected to 110,000 G for 24 hours show a marked depression in their growth characteristics. Ultrastructural studies of these bacteria have demonstrated that this failure of growth is in reality a failure of division. When bacteria are exposed to 110,000 G for 24 hours the ultrastructural characteristics show marked variations from normal. The most outstanding of these variations is a general enlargement of the cell and the intracellular components. Preliminary investigations reveal that these gravitational changes may be of a genetic nature. This disassociation of growth and division can be produced in biologic specimens by X-radiation. Bacteria subjected to 24 hours of continuous irradiation from a cobalt bomb source which delivers 2 R per minute, show ultrastructural alterations which appear to be identical to those induced by gravity. Studies are in progress to evaluate the growth characteristics of these irradiated bacteria and to establish whether

or not these ultrastructural alterations are of a genetic nature. Following the completion of these observations a metabolic study of both groups of bacteria will be initiated.

B. The centrifuge microscope has been used to study the growth characteristics and the functional morphologic characteristics of amoebae proteus. The growth rates of the amoebae do not appear to be influenced by subjecting them continuously to 20 G or 40 G for as long as five weeks. At both levels of gravity the amoebae have been photographed and their motion characteristics studied. The principal alteration of motion consists of a jerkiness in the extension of the normally freely flowing pseudopods. The heavy particulate material in the amoebae clusters at one end. The clustering of this material does not appear to adversely affect the amoebae. At 40 G the amoebae may be seen to trap paramecia in the usual fashion. These studies will be continued by raising the G force to approximately 100 G.

C. The equipment for the inflight studies involving zero gravity and zero gravity plus X-radiation has been thoroughly studied and tested during the summer. The optimum number of cells per culture chamber has been established for cell growth during a 21 day period. The optimum media mixture for cell growth during a 21 day period has been established. The only technical problem in this aspect of the experiment is the development of a method for the prevention of the accumulation of gas bubbles wihtin the culture chamber. These studies are in progress at the present time. The exact nature of the physical arrangement required for the inflight instrumentation studies will be pursued in the near future. It is expected that the inflight equipment will be completely fabricated prior to the summer of 1964. This equipment will be used for the 21 day inflight zero gravity study and the 3 day zero gravity plus radiation study.

III. Radiation Studies

The effects of 600 R of X-radiation have been thoroughly investigated in Chang liver cells. The data are composed of time lapse motion picture observations of the phase contrast images of the living cells for up to one week after X-radiation. Ultrastructural studies of the cell organelles have demonstrated extensive changes in many of the membranous components of the cells as well as in such non-membranous components as the nucleoli. A major feature of the effect of this level of X-radiation on cells is the disassociation of cell growth from cell division. This results in the production of single giant cell forms and multinucleated giant cell forms. These data will be used as control material for the inflight studies of effects of zero gravity on cells subjected to X-radiation.

As mentioned above the effects of constant low dosage of radiation on bacteria are being compared with the effects of increased gravity on bacteria.

IV. Equipment Development

The ultraviolet flying spot television microscope has been reassembled and at the present time is being used to study the ultraviolet absorption characteristics of protein microspheres in collaboration with Dr. Sidney Fox. The object of the experiment is to determine whether or not these microspheres have true membranes. A new amplifier has provided the instrument with improved signal to noise ratios and more stability. Ultraviolet emitting phosphors have not changed appreciably since the previous progress report.

The development of the stepping spot microscope has reached a plateau because of the inherent limitation of signal to noise ratios. In the future attempts will be made to adapt this principle to lasers and fiber optics and to film scanning.

The equipment for the ultraviolet microbeam closed circuit phase contrast television studies has been moved, reassembled and put into working condition. The experiments outlined in the grant are in progress utilizing this equipment.

Due to the fact that the inflight studies will be made in recoverable satellites no other studies of the potential inflight use of a miniaturized vidicon system have been made and at the present time no further studies of this nature are contemplated.

The present centrifuge microscope has been modified to permit observations of amoebae at up to 100 G. Considerable preliminary thought has been given to the design parameters of a centrifuge microscope for the observation of human tissue culture cells. At the present time plans are to develop a vidicon centrifuge microscope for this purpose. This instrument will permit us to obtain data on the behavioral characteristics of human cells while they are subjected to relatively small increments of gravity for prolonged periods of time. Such data are not now available and will be valuable in the evaluation of the overall nature of gravitational effects on human material.